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Relationship between Cytotoxic Activity and Dipole Moment for Phthalimido- and Chloroethyl-Phenothiazines

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Abstract. *Among twelve phenothiazine-related compounds, the cytotoxic activity of six "half-mustard type" phenothiazines [**7-12**] was significantly higher than that of six phthalimido compounds [**1-6**]. 1-(2-Chloroethyl)-3-(2-chloro-10H-phenothiazin-10-yl)propylurea [**9**], 1-(2-chloroethyl)-3-(2-chloro-10H-phenothiazin-10-yl)butylurea [**10**] and 1-(2-chloroethyl)-3-(2-trifluoromethyl-10H-phenothiazin-10-yl)butylurea [**12**] showed the highest cytotoxic activity, in parallel with high $\Delta\mu$ (difference between two dipole moments, μ_g and μ_e). There was also positive relationship between cytotoxic activity and molecular orbital energy such as π-LUMO, π-HOMO, and lone pair orbitals originated from O, N1, and N3 atoms. The present study demonstrated that cytotoxic activity of "half-mustard type" phenothiazines can be predicted by their dipole moments and molecular orbital energies.*

Benzo[*a*]phenothiazines, which have differentiation-inducing activity and antitumor activity, showed a significantly smaller value of ground-state dipole moment (μ_g) and larger value of first excited-state dipole moment (μ_e), as compared with inactive compounds (1). The μ_g and μ_e of "half-mustard type" phenothiazines have also been investigated by MOPAC program (version 6.01) (2). On the other hand, the possible role of π-electron density (ϱ^{π}) or radical generation for expression of biological activity has been discussed (3). Both μ_g and spectral data of benzo[*a*]phenothiazines were used to evaluate their μ_e by the solvatochromic shift method (Bakhshiev and Kawski-Chamma-Viallet correlations) (4). Since the dipole moment of a molecule is defined as the sum of vector of several interdependent moments originating from various parts of the molecule. Therefore, a satisfactory agreement was obtained in most cases where the experimental μ_e were higher than their μ_g (4). These quantum-chemical calculations such as two dipole moments (μ_g, μ_e), ϱ^{π} and radical intensity might be useful parameters to predict biological activity. The purpose of this paper was to clarify the possible relationship between biological activity and calculated electronic properties such as dipole moments and molecular orbitals of the unshared electron pair in the urea moiety of phthalimido- and chloroethyl-phenothiazines.

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Materials and Methods

Synthesis of chemicals. Twelve phenothiazines [**1-12**] were prepared as described previously (5).

Cytotoxic activity. A 50% cytotoxic concentration (CC_{50}) against human myelogenous leukaemic cell lines was tested as described previously (6).

Dipole moments and molecular orbital energy. The molecular orbital was calculated by parametric method 3 (PM3), using the MOPAC program (Version 6.01) (7). The optimized geometrical structures of compounds [**1-12**] were obtained and their geometric and electric changes were investigated. The dipole moments of the first excited-state which consists of one triplet and three singlet states were calculated with MECI algorithm. For this calculation, the FACOM M770 computer and HP-7000 EWS computer in the Josai University Information Sciences Center were used.

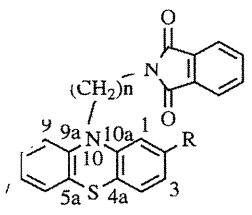
Results and Discussion

Relationship between dipole moments and biological activities. Table I summarizes the cytotoxic activity, dipole moment and molecular orbital energy of "half-mustard type" phenothiazines. The cytotoxic concentrations (CC) of the compounds in mg/mL can be compared easily.

Relationship between dipole moments and cytotoxic activity. The dipole moment usually reflected the interaction of intermolecular dipoles. Then, twelve μ_g of [**1-12**] and six μ_e of [**7-12**] were calculated by PM3 method (8) (Table I).

Six phthalimido compounds [**1-6**] showed weak cytotoxic activity (CC_{50} > 400 μg/mL), with μ_g values of 4.03 D, 4.92 D,

Table I. *Effect of dipole moments and molecular orbital energies of six "half-mustard" phenothiazines [7-12] on the proliferation of HL-60 cells.*



[1], R=H, n=3
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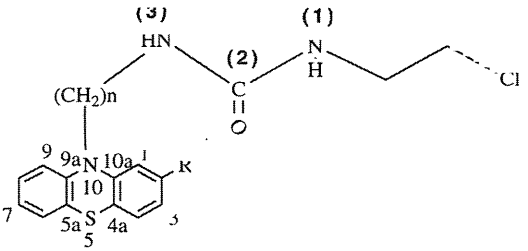
[3], R=Cl, n=3
[4], R=Cl, n=4
[5], R=CF_3, n=3
[6], R=CF_3, n=4



[7], R=H, n=3
[8], R=H, n=4
[9], R=Cl, n=3
[10], R=Cl, n=4
[11], R=CF_3, n=3
[12], R=CF_3, n=4

Compd's	Cytotoxic activity	Dipole moment[b]			Molecular orbital energy (by PM3)					
No.	(CC_{50}) (μg/mL)[a]	μ_g[c]	μ_e[d]	$\Delta\mu$[e]	π-HOMO	π-LUMO	ΔE[f]	n_o[g]	n_a[h]	n_b[i]
7	35	2.63	3.08	0.45	-8.046	-0.328	7.718	-11.117	-9.991	-9.654
8	38	4.45	4.00	0.45	-7.890	-0.291	7.599	-11.150	-10.017	-9.680
9	6	3.02	1.08	1.94	-8.073	-0.500	7.573	-11.215	-10.082	-9.757
10	1 0	3.57	3.64	0.07	-7.983	-0.476	7.507	-11.158	-10.023	-9.692
11	4	2.99	8.20	5.21	-8.261	-0.911	7.350	-11.225	-10.085	-9.760
12	5	5.05	11.59	6.54	-8.316	-0.858	7.458	-11.180	-10.022	-9.806

[a] ref. 6. [b] ref. 8. [c]μ_g: ground-state dipole moment (Debye unit). [d]μ_e: first excited-state dipole moment (Debye unit). [e]$\Delta\mu$ $|\mu_g-\mu_e|$ (Debye unit). [f]ΔE: energy gaps (HOMO-LUMO). [g]n_o: lone pair orbital due to O atom. [h]n_a: lone pair orbital due to N1, O and N3 atoms. [i]n_b: lone pair orbital due to N1 and N3 atoms.

3.34 D, 4.59 D, 4.95 D and 4.62 D, respectively.

The magnitude of the ground state dipole moments (μ_gs) and magnitude of the first-excited state dipole moments (μ_es) ranged from 2.63 D to 5.05 D, and from 1.08 D to 11.59 D, respectively. Compounds **9**, (CC_{50} = 6 μg/mL; Δμ:1.94 D), **11**, (CC_{50} = 4 μg/mL; Δμ:5.21 D), and **12**, (CC_{50} = 5 μg/mL; Δμ:6.54 D) showed higher cytotoxic activity and Δμ than those of compounds **7** (CC_{50} = 35 mg/mL; Δμ:0.45 D), **8** (CC_{50} = 38 mg/mL; Δμ:0.45 D), and **10** (CC_{50} = 10 mg/mL; Δμ:0.07 D). This suggests a positive relationship between Δμ and cytotoxic activity (6, 8) (Table I).

Relationship between cytotoxic avtivity and π-HOMO and LUMO energies. Selected molecular orbital energies for six "half-mustard type" phenothiazines [**7-12**] are listed in Table I. Compounds with higher π-HOMO or π-LUMO energy ([**9**] (π-HOMO = -8.073 eV; π-LUMO = -0.500 eV; CC_{50} = 6 μg/mL), [**11**] (π-HOMO = -8.261 eV; π-LUMO = -0.911 eV; CC_{50} = 4 μg/mL), and [**12**] (π-HOMO = -8.316 eV; π-LUMO = -0.858 eV; CC_{50} = 5 μg/mL) were more cytotoxic than compounds with lower π-HOMO or π-LUMO energy, [**7**] (π-HOMO = -8.046 eV; π-LUMO = -0.328 eV; CC_{50} = 35 μg/mL), [**8**] (π-HOMO = -7.890 eV; π-LUMO = -0.291 eV; CC_{50} = 38 μg/mL), and [**10**] (π-HOMO = -7.983 eV; π-LUMO = -0.476 eV; CC_{50} = 10 μg/mL). This suggests the positive relationship between cytotoxic activity and π-HOMO or π-LUMO energy (6) (Table I). The difference (ΔE) between ground state and excitability represented by molecular stability indices such as π-HOMO and π-LUMO was generally smaller in highly cytotoxic compounds [**9, 11, 12**] (6) (Table I). The finding suggests that the binding of the compounds is rather specific and, therefore requires relatively low energies.

Orbital energies of unshared pair electron on urea site. The n_a orbital is a lone pair orbital related to N1, O and N3 atoms on the urea site. The n_o orbital is a lone pair orbital of O atom on the site. The n_b orbital is a lone pair orbital of N1 and N3 atoms on the site. A similar positive relationship was found between CC_{50} and a lone pair orbital energy (n_a, n_b and n_o orbital energies) (6) (Table I). Active compounds [**9, 11, 12**] had a more stable orbital energy than the less active compounds [**7, 8, 10**].

Multiple regression analysis between CC_{50} and electronic structure on urea site. In order to obtain a more quantitative correlation between CC_{50} activity and electronic property on the urea site, the coefficients were calculated by multiple determination and Fisher's statistic values (F) (6) (Table I). The multiple correlation coefficient (r^2) between CC_{50} and the model based on four parameters due to the orbital energies of n_a, n_b, n_o and net atomic charge of the O atom, was calculated to be 0.83. However, F for this model (1.19) was much smaller than that of the five percent critical value F (4, 1; 0.05) = 225; thus, this model seems unclear. The multiple correlation coefficient (r^2) between CC_{50} and two orbital energies based on n_b and n_o was 0.75. But, F (4.39) was smaller than F (2, 3; 0.05) = 9.55 (6) (Table I).

An urea portion of "half-mustard type" phenothiazines showed significant variability in the energy of lone pair orbital of N1, O, and N3 atoms (6) (Table I).

In conclusion, the present study demonstrates that cytotoxic activity of "half-mustard type" phenothiazines can be predicted by their dipole moments and well defined molecular orbital energies.

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